

AMENDMENTS TO THE SPECIFICATION

Please amend the paragraph at page 27, lines 4-13, as follows:

As specific examples of the hybridoma cell lines of the present invention, hybridoma cell lines KM2311, KM2582, KM2604, KM2590, and KM2591 can be mentioned. Hybridoma cell line KM2311 was deposited with the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology (1-3, Higashi 1 chome Tsukuba-shi, Ibaraki-ken, JAPAN) as FERM BP-6306 on March [[23]] 24, 1998; hybridoma cell lines KM2382 and KM2604 were deposited with the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology as FERM BP-6663 and FERM BP-6664, respectively, on February 26, 1999; hybridoma cell lines KM2590 and KM2591 were deposited with the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology as FERM BP-6683 and FERM BP-6684, respectively, on March 19, 1999.

Please amend the paragraph at page 48, lines 11-17, as follows:

The hTERT protein sequence was analyzed using Genetyx Mac, and a partial peptide of 1-17 from the N-terminal of the human telomerase catalytic subunit (Compound [[1]] 2, SEQ ID NO: 1), a partial peptide of 642 - 661 from the N-terminal of the human telomerase catalytic subunit (Compound [[2]] 1, SEQ ID NO: 2), and a partial peptide of 1177 -1192 from the N-terminal of the human telomerase catalytic subunit (Compound 3, SEQ ID NO: 3) were selected from the highly hydrophilic sections, the N-terminal and the C-terminal, as partial sequences considered to be suitable as antigens.

Page 49, after line 22, insert the following text:

Ac: acetyl

Please amend the paragraph at page 51, line 21 to page 52, line 17, as follows:

Next, H-Lys(Boc)-Ala-Cys(Trt) was synthesized on a carrier by conducting a condensation reaction using the Fmoc-Lys(Boc)-OH using the process of (a), and by passing through the washing process of (b), and the protection removal processes of (c) and (d). Next, a carrier resin bound to a side chain protection peptide was obtained by successively using Fmoc-Val-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Ser(t-Bu)-OH, Fmoc-Thr(t-Bu)-OH, Fmoc-Leu-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Glu(Ot-Bu)-OH, Fmoc-Ala-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(Ot-Bu)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Phe-OH, Fmoc-Thr(t-Bu)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Ala-OH, then, after repeating (a) - (d), washing successively with methanol and butylether and drying under reduced pressure for 12 hours. To this, 1 ml of a solution mixture comprising TFA (82.5%), theoanisol (5%), water (5%), ethyl methyl sulfide (3%), 1,2-ethanedithiol (2.5%) and thiophenol (2%) was added and allowed to stand for 8 hours at room temperature, and the side chain protection units were removed while the peptide was separated from the resin. After filtering the resin, approximately 10 ml of ether were added to the obtained solution, the produced precipitate was collected by centrifugation and decantation, and thereby 36.2 mg were collected as crude peptide. After washing this crude product in 2 M acetic acid, it was purified by HPLC using a reverse phase column (CAPCELL PAK C18 30 mmI.D. X 25 mm, manufactured by Shiseido). The fraction containing Compound 1 was obtained by elution using linear gradient method carried out with the addition of 90% acetonitrile aqueous solution containing 0.1 % TFA to a 0.1 % TFA aqueous solution, and detection at 220 nm. 2.3 mg of Compound [[2]] 1 was obtained by freeze drying this fraction.

Please amend the paragraph at page 52, line 23 to page 53, line 8, as follows:

A carrier resin bonded with side chain protective peptides was obtained using 30 mg of a carrier resin (chlorotriyl resin manufactured by AnaSpec) bonded to 14.1 μ mol of H-Cys(Trt) as the starting material, in the same way as in Example 1, by successively condensing Fmoc-Ser(t-bu)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Ser(t-Bu)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Val-OH, Fmoc-Ala-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Ser(t-Bu)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Pro-OH, Fmoc-Ala-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Pro-OH, and Fmoc-Met-OH, washing and drying. In the same way as in Example Compound 1, cleavage of side chain protection groups and separation from the resin were carried out, 31.1 mg of crude peptide was obtained, and purified by HPLC using a reverse phase column, thereby, 4.8 mg of Compound [[1]] 2, was obtained.

Please amend the paragraph at page 55, lines 4-18, as follows:

In the antigen for the assay, one in which the hTERT partial peptide obtained in Example 1(1) was conjugated with thyroglobulin (hereinafter abbreviated as THY) was used. The preparation method was the same as described in Example 1(2), except that SMCC (Sigma Co.) was used in place of MBS as the cross-linking agent. To a 96 well EIA plate (Griener Co.), 50 μ l of the 10 μ g/ml conjugate prepared in the above mentioned way was added to each well and allowed to stand over night at 4°C to adsorb. After washing, 100 μ l of 1% BSA-PBS was added to each well, reacted for 1 hour at room temperature, and the remaining active groups blocked. The 1% BSA-PBS was discarded, and 50 μ l of antiserum of the mouse to be immunized, culture supernatant of the anti-hTERT monoclonal antibody or purified monoclonal antibodies were added to each well and allowed to react for 2 hours. After washing with tween - PBS, 50 μ l of rabbit anti-mouse immunoglobulin labeled with

peroxidase (Dako Co.) was added to each well and allowed to react for 1 hour at room temperature, then, after washing with tween - PBS, color was developed using ABTS matrix liquid [2,2-adenobis(3-ethylbenzothiazol-6-sulfonic acid) ammonium] and OD415 nm absorbance was measured using a plate reader (NJ 2001; manufactured by Nihon Intermed Co.)

Please amend the paragraph at page 59, line 8 to page 60, line 3, as follows:

Five types of cells, human renal transformant 293 (ATCC CRL1537), human cervical cancer cell line HeLaS3 (ATCC CCL - 2.2), human colon cancer cell line CoLo205 cell (ATCC CRL - 225), normal human lung cells MRC5 (ATCC CCL -171), and normal human lung cells WI-38 cell (ATCC CCL-75), were used. Cells of these lines were floated in a trypsin and EDTA solution mixture (Sankou Junyaku) and washed in PBS. 1 ml of buffer for cytolysis (50 mM Tris-HCL, pH [[7.]] 7.2, 1% TritonX, 150 mM NaCl, 2mM MgCl₂, 2mM CaCl₂, 0.1% NaN₃, 50mM iodoacetamide, 50 mM N-ethylmaleimide, 1 mg/ml leupeptin, and 0.1 mM dithiothreitol) was added to 5 x 10⁷ cells, and allowed to stand for 2 hours at 4°C, and then centrifuged. After fractionation of the obtained supernatant by SDS-electrophoresis with 10⁵ cells per lane (Antibodies - A Laboratory Manual, Cold Spring Harbor Laboratory, 1988), blotting to a PVDF membrane was carried out. After blocking with BSA-PBS, the culture supernatant of the anti-hTERT monoclonal antibodies ~~were~~ was allowed to react for 2 hours at room temperature. After washing wells with PBS-Tween, a reaction with anti-mouse immunoglobulin antibody (manufactured by Dako. Co.) labeled with peroxidase as the second antibody was carried out for 1 hour at room temperature. After washing well with PBS - Tween, detection was carried out using an ECL detection kit (Amersham Co.), and sensitized on X-ray film. The results are shown in Figure 4. In Figure

4, Lane 1 shows the results for the 293 cytolysis solution, Lane 2 shows the results for the HeLaS3 cytolysis solution, Lane 3 shows the results for the CoLo205 cell cytolysis solution, Lane 4 shows the results for the MRC 5 cytolysis solution, and Lane 5 shows the results for the WI-38 cell cytolysis solution.

Please amend the paragraph at page 60, lines 15-17, as follows:

Detection of hTERT protein within cells by dot blotting was studied using anti-hTERT monoclonal antibodies. As the anti-hTERT monoclonal antibodies, the culture supernatant of KM2311 detected hTERT protein in Example 1 (7) was used.

Please amend the paragraph at page 62, line 20 to page 63, line 11, as follows:

First, synthetic DNA primers were produced using a gene portion corresponding to 549 to 831 amino acid residues (SEQ ID NO: 6) [Science, 277, 955 (1997)] of hTERT which have total 1132 amino acid residues, as a probe, respectively. SEQ IS NO: 4 is a nucleotide sequence in which a recognition sequence by BamHI is combined to 5' terminal of a nucleotide sequence corresponding to 439 to 555 amino acid residues. SEQ ID: 5 is a nucleotide sequence in which a recognition sequence by EcoRI is combined to the 5' terminal of a nucleotide sequence corresponding to 825 to 831 amino acid residues. The DNA was amplified by means of PCR using these synthetic DNA primers. The reaction conditions were as follows: after allowing to stand for 1 minute at 94°C, repeating 25 cycles consisting of 20 seconds at 94°C, 30 seconds at 55°C, and 2 minutes at 72°C; then, allowing to stand at 72°C for 10 minutes; and shifting to 4°C. As thermal resistant DNA polymerase, LA Taq DNA polymerase manufactured by Takara Co. was used. The obtained PCT product was digested with restriction enzymes BamHI and EcoRI, the sample was subject to

electrophoresis in agarose gel, the DNA band was cut out, and thereby the DNA was extracted and purified. The purified fragment was inserted into BamHI-EcoRI site in pGEX-2TK (manufactured by Pharmacia Co.), in which the DNA coding for GTS was incorporated, and the obtained plasmid was named pHERT.

Please amend the paragraph at page 67, line 14 to page 68, line 8, as follows:

To a 96 well EIA plate (Griener Co.), 50 μ l of the 4 μ g/ml anti-rat immunoglobulin antibody (mouse antibody absorption finished; manufactured by Cartag Co.) ~~were~~ was added to each well and allowed to be adsorbed onto the plate by allowing to stand over night at 4°C. After washing, 100 μ l of 1% BSA-PBS was added to each well, reacted for 1 hour at room temperature, and the remaining active groups blocked. The 1% BSA-PBS in the wells was discarded, each of undiluted culture supernatant of the hybridomas KM2590 and KM2591 was added each well and allowed to react ~~for 24 hours over night~~ at 4°C. After washing the wells with PBS, a series of five-fold dilution was repeated seven times with respect to each of insect cell nucleus extracts of cells expressing the hTERT protein and the insect cell nucleus extracts of cells expressing only a vector, and each of the diluted extracts was added to each well, and allowed to react over night at 4°C. After washing the wells with tween-PBS, 50 μ l of the above-mentioned KM2311 labeled with biotin (diluted in BSA-PBS containing 1% normal rat serum to 1 μ g/ml) was added to each well, and allowed to react for 2 hours at room temperature. Then after washing the wells with tween-PBS, 50 μ l of avidin labeled with peroxidase (Vector Co.) was added to each well, and allowed to react for 1 hours at room temperature. Then, after washing the wells with tween-PBS, the color was developed using ABTS matrix liquid [2,2-azinobis (3-ethylbenzothiazole-6-sulfonic acid) ammonium]

and OD415 nm absorbance measured using a plate reader (E-max; manufactured by WAKO JUNYAKU).

Please amend the paragraph at page 68, line 18 to page 69, line 7, as follows:

To a 96 well EIA plate (Griener Co.), 100 µl of anti-rat immunoglobulin (manufactured by Cartag Co.) were added to each well and allowed to stand over night at 4°C to be coated on the plate. Then, 200 µl of BSA-PBS were added to each well, allowed to stand for 1 hour at room temperature, and the remaining active groups were blocked. Then, the BSA-PBS in the wells was discarded, 100 µl of undiluted culture supernatant of the control antibody KM844, the hybridoma KM2590, or KM2591 were added respectively to each well and allowed to react for 2 hours at room temperature. After washing thoroughly with PBS, 100 µl of the cell extract was added to each well and allowed to react ~~for 2 hours~~ over night at 4°C. After washing thoroughly in wells with PBS - tween, 20 µl of sample buffer (five fold concentration) for SDS-PAGE was added to each well, the plate was shaken for 2 hours, and all contents were poured into tubes. The collected samples were diluted five times PBS, and according to a usual method, SDA-PAGE was performed, followed by Western blotting. Then, the antibody staining was carried out using the anti-hTERT monoclonal antibody KM2311 obtained in Example 1.